



## The taxonomy of the Japanese oak red scale insect, *Kuwania quercus* (Kuwana) (Hemiptera: Coccoidea: Kuwaniidae), with a generic diagnosis, a key to species and description of a new species from California

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### Abstract

The oak red scale insect, *Kuwania quercus* (Kuwana), was described from specimens collected from the bark of oak trees (*Quercus* species) in Japan. More recently, the species has been identified from California and China, but Californian specimens differ morphologically from Japanese material and are considered here to be a new species based on both morphological and molecular data. In this paper, an illustrated redescription of *K. quercus* is provided based on type specimens consisting of adult females, first-instar nymphs and intermediate-stage females, and a lectotype is designated for *Sasakia quercus* Kuwana. The new Californian species, *Kuwania raygilli* Wu & Gullan, is described and illustrated based on the adult female, first-instar nymph and intermediate-stage female. A new generic diagnosis for *Kuwania* Cockerell based on adult females and first-instar nymphs, and a key to species based on adult females are included.

**Key words:** *Kuwania quercus*, *K. bipora*, *K. raygilli*, new species, lectotype

### Introduction

The genus *Kuwania* Cockerell (*in* Fernald, 1903) as currently understood, is mostly Palaearctic and Oriental in distribution and contains six species: *K. bipora* Borchsenius from *Castanopsis* and *Quercus* (Fagaceae), *K. minuta* Borchsenius also from *Quercus*, *K. oligostigma* De Lotto from *Commiphora* (Burseraceae), *K. pasaniae* Borchsenius from several genera of Fagaceae, *K. quercus* (Kuwana) from *Quercus* except one Californian record from *Castanea* (Fagaceae), and *K. rubra* Goux, also from *Quercus* (Ben-Dov, 2005; Wang & Wang, 1986; Wu, 2008). *Kuwania betulae* Borchsenius was synonymised with *Xylococcus japonicus* Oguma by Danzig (1972), and two other species that previously were placed in *Kuwania* by some authors are now in *Neogreenia* MacGillivray, as *N. zeylanica* (Green) and *N. osmanthus* (Yang & Hu) (Yang & Hu, 1994; Wu & Cheng, 2006; Wu & Nan, 2012). All female and male instars of *K. bipora* have been described recently (Wu, 2008), but the other species are poorly known taxonomically and there is variation in the morphology of the type species *K. quercus* in different parts of its reported range.

*Kuwania quercus* was described based on specimens collected from Tokyo and Kyushu Island in Japan on the bark of oak trees, *Quercus* spp. (Kuwana, 1902). Originally this species was named *Sasakia quercus* Kuwana and was the only species in Kuwana's new genus, *Sasakia*. Cockerell (*in* Fernald, 1903) found that this genus name had been preoccupied by *Sasakia* Moore, 1896, in the family Nymphalidae (Lepidoptera), and replaced Kuwana's genus name with *Kuwania*. The description and illustrations in Kuwana (1902) are very simple, especially lacking in microscopic detail, and cannot be used for correct identification. Ferris (1919) provided further details as well as basic line drawings of the first-instar nymph, an intermediate female nymph and several enlargements of microscopic features, based on preparations from Kuwana's type material. Since then, this species has been described and illustrated by Morrison (1928) based on specimens collected in 1926 from an unspecified locality,

presumably in Japan; by Ferris (1950) based on Chinese specimens; by Paik (1978) based on Korean specimens; by Kawai (1980) based on Japanese specimens, and by Gill (1993) based on specimens from California, USA. However, there are differences among these descriptions and illustrations, mainly in number of abdominal spiracles and the types and structure of the disc pores, and it seems that more than one species is involved. Gill (1993) reported that the specific status of the Californian form was suspect because J.W. Beardsley had noted differences between the Californian and Asian collections. From Morrison's description and illustration, we also believe that the adult specimens he used are not conspecific with those of Kuwana (1902), because Morrison's specimens had six pairs of abdominal spiracles and no discoidal pores on the abdominal venter, whereas *K. quercus* has four pairs of abdominal spiracles and ventral discoidal pores on the abdomen.

In Japan, the adult females of *K. quercus* are typically found in crevices in the bark and produce a white cottony secretion (Kuwana, 1902) that acts as an ovisac into which eggs are laid (Kawai, 1980). The live adult female and nymphs of all instars are red in colour and mature females are only 1.5–2.0 mm long (Kuwana, 1902; Kawai, 1980). Kawai (1980) aptly referred to this species as the 'oak red scale insect'. As with *K. bipora* (Wu, 2008), the female life history consists of a first-instar nymph, two apodous (legless) feeding instars and the fully-legged adult female (Ferris, 1950); males are not known. Kawai (1980) describes the life cycle in Japan as univoltine, with newly hatched nymphs appearing near the end of May (late spring) and settling in groups in bark crevices. When large populations are present, the nymphs apparently burrow under the bark, causing it to develop a rough texture (but see below concerning waxen cells). Overwintering occurs in the apodous nymphal stage and, from late April until June, adult females emerge and wander seeking oviposition sites. Large populations are easily noted because the trunk and branches of the host oak trees become white with the ovisacs. Kawai (1980) records *K. quercus* as common in the Kanto region of northern Japan through the Kinki region of central Japan, with outbreaks often seen in Tokyo, but with the abundance declining towards the south, being a relatively rare species in Okinawa. He also mentioned a much larger, undescribed species from 'red oak' (host plant listed as 'Akagashi'), but did not provide any further details.

Kawai (1980) did not state whether the intermediate-stage females produced a waxen cell or test, but his colour photograph (figs 2–7c) of these nymphs on the stem of an oak shows bumps that might be secretions produced by the insects. In California, individuals identified as *K. quercus* usually hide in or under the bark, especially under rough loosened bark (Gill, 1993; PJG, personal observation), and the apodous preadult females are covered by a hard white to greyish waxen cell that blends with the colour of the bark (Gill, 1993). Ferris (1950), who collected *K. quercus* from *Q. aliena* in Yunnan Province in China, also described small waxen cells containing the apodous nymphal females and, in contrast to infestations in Japan, the insects occurred on the smaller limbs and twigs of the trees without concealment in cracks on the bark. Takahashi (1934) recorded *K. quercus* from Taiwan, China, stating that in late April only eggs and dead adult females were present on the stems and branches of two *Quercus* species. Kanda (1930) described the male of *K. quercus*, but it is obviously the male of a species of mealybug.

The aims of this paper are to redescribe *K. quercus* and provide modern illustrations based on Kuwana's type series, to designate a lectotype, and to determine the identity of Californian specimens previously identified as *K. quercus*. We also provide a revised diagnosis of *Kuwania* based on adult females and first-instar nymphs and a key to species based on adult females.

## Materials and methods

**Morphological work.** Type specimens of *Sasakia quercus* were borrowed from the following museum collections: The Bohart Museum of Entomology (BME), University of California, Davis, California, USA; the United States National Collection of Coccoidea of the National Museum of Natural History (USNM), Smithsonian Institution, housed at the United States Department of Agriculture (USDA), Beltsville, Maryland, USA. We also examined specimens of *K. bipora* collected on *Castanopsis fissa* in Guangdong Province in China (Wu, 2008) and housed in the insect collection of the Department of Forestry Protection, Beijing Forestry University, Beijing, China (BFUC), and specimens of putative *K. quercus* from *Q. lobata* in Davis, California, USA, collected by PJG in July 2001 (housed in the Australian National Insect Collection, Canberra, Australia (ANIC), BJUC, BME and USNM) and R.J. Gill in May 2012 (housed in the BFUC and BME). Freshly collected specimens of *K. quercus* were sent from

Japan (ex *Q. myrsinifolia* from Osaka, Honsyu Island) by Hirotaka Tanaka (housed in BFUC). The identification key was constructed based on examination of the above specimens as well as the descriptions and illustrations in Goux (1938), Borchsenius (1955), De Lotto (1959) and Danzig (1980).

Morphological examinations were made with an Olympus BX40 compound microscope and a Leica DME compound microscope. Illustrations were prepared using a Leica drawing tube and the 100X objective with oil immersion was required to see details of the cuticular pores and setae. The morphological terms of Morrison (1928) and Wu (2008) were used for most structures, except that terms for antennal sensilla are from Koteja (1980). Thus, the larger stout sensilla towards the antennal apex are thin-walled pegs that are usually referred to as fleshy setae (but if longer, then they are often called antennal bristles), and the sensory pores on segment II are called campaniform sensilla. Measurements were based on the number of specimens specified in the description of each instar.

**Molecular work.** We also conducted a molecular study in an attempt to resolve the identity of the Californian specimens of *Kuwania*. DNA was extracted and amplified from adult females of the Californian population (ex *Q. lobata*, Davis; referred to as *Kuwania* sp. CA in the two tables), of *K. quercus* from Japan and of *K. bipora* from China (Table 1) using DNeasy Blood & Tissue Kit (Qiagen) in accordance with the manufacturer's protocols. Amplification of COI and 18S were performed in 50 µl reactions using the respective primer pairs: LCO1490 (5' -GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5' -TAAACTTCAGGGTGACC AAAAAATCA-3') (Folmer *et al.*, 1994), and 2880 (5' -CTGGTTGATCCTGCCAGTAG) (Tautz *et al.*, 1988) and B- [rev] (5' -CCGCGGCTGCTGGCACCAGA) (von Dohlen & Moran, 1995). For COI, reactions contained 4 µl DNA template, 5µl 10xBuffer, 25 mM MgCl<sub>2</sub>, 2.5 mM dNTP mixture, 10 pmol of each primer and 1 unit of ExTaq DNA polymerase (TaKaRa Bio Inc., Dalian, China). PCRs were run on Eppendorf Mastercycler Thermal Cyclers with the following profile: an initial step of 1 min at 94 °C; five cycles of 1 min at 94°C, 1.5 min at 45°C and 1.5 min at 72°C; 35 cycles of 1 min at 94°C, 1.5 min at 50°C and 1 min at 72°C and a final cycle of 5 min at 72°C. For 18S, each reaction contained 25 µl 2×MightyAmp Buffer Ver.2, 15 pmol each of the primers, 5 µl DNA template and 1 unit of MightyAmp DNA Polymerase (TaKaRa Bio Inc., Dalian, China). PCR cycles were as follows: an initial 2 min denaturing at 98 °C; 34 cycles of 10 s at 98°C, 15 s at 60°C and 1 min at 68 °C; followed by 5 min at 68 °C. Products were visualized on 1% agarose, and the most intense products were sequenced bidirectionally using BigDye v3.1 on an ABI3730xl DNA Analyzer (Applied Biosystems). Sequences of the two gene fragments were aligned in Bioedit (Hall, 1999). The sequences of *Kuwania* species obtained in this study were deposited in the GenBank under the accession numbers KC417337—KC417342. The pairwise genetic divergences were calculated using the K2P distance model (Kimura, 1980).

**TABLE 1.** Collection information and GenBank accession numbers for *Kuwania* spp. used in this study.

Species name	Life stage	Host tree	Location collected	Date collected	Collector	GenBank Accession No. 18S	GenBank Accession No. COI
<i>Kuwania</i> sp. CA	Adult female	<i>Quercus lobata</i>	USA: California, Yolo County, Davis	2 May 2012	R.J. Gill	KC417337	KC417340
<i>K. quercus</i>	Adult female	<i>Quercus myrsinifolia</i>	JAPAN: Osaka, Honsyu Island	3May 2012	Hirotaka Tanaka	KC417338	KC417341
<i>K. bipora</i>	Adult female	<i>Castanopsis fissa</i>	CHINA: Guangdong, Guangzhou	20 October 2012	Shao-Bin Huang	KC417339	KC417342

## Systematics

### Californian specimens previously identified as *K. quercus*

The illustration by Gill (1993) of the Californian adult female identified as *K. quercus* is more similar to *K. bipora* than to *K. quercus*, the main difference being the number of outer loculi in the two types of multilocular disc

pore. These loculi are difficult to see and count, even using the 100X objective under oil immersion, because of their small size and the concavity of the pores. Also, the number of loculi in these disc pores varies and so it is difficult to determine whether this Californian species is conspecific with *K. bipora*, based only on morphological characters. The molecular study helped to resolve this problem.

The pairwise genetic divergences in COI among *K. quercus*, *K. bipora* and *K. sp. CA* are 9.3–11.5% (Table 2), which is a much greater range than <2% reported for the closely-related mealybugs *Planococcus citri* (Risso) and *Pl. minor* (Maskell) (Rung *et al.*, 2008) and <7.7% for species in the genus *Ferrisia* (Lin *et al.*, 2013). These distances are comparable to the average of 10.1% among congeneric species in the families Pseudococcidae and Diaspididae (Park *et al.*, 2011), and to approximately 12.3% for *Ceroplastes* species of family Coccidae (Deng *et al.* 2012). The pairwise genetic divergence in 18S between the Californian sample and *K. bipora* is 4.7%, slightly greater than that (4.1%) between *K. bipora* and *K. quercus*. The above results indicate that the Californian population should be recognised as a separate species, and is neither *K. bipora* nor *K. quercus*. Below we name, describe and illustrate this new species based on all available life stages.

**TABLE 2.** Pairwise genetic divergences among three *Kuwanina* species.

	COI			18S		
<i>Kuwanina</i> sp. CA	0			0		
<i>K. quercus</i>	0.093	0		0.020	0	
<i>K. bipora</i>	0.113	0.115	0	0.047	0.041	0

## Genus *Kuwanina* Cockerell in Fernald

*Sasakia* Kuwana, 1902: 47. Type species: *Sasakia quercus* Kuwana, by monotypy. Homonym of *Sasakia* in Lepidoptera; discovered by Fernald, 1903: 32.

*Kuwanina* Cockerell in Fernald, 1903: 32. Replacement name for *Sasakia* Kuwana, 1902.

**Diagnosis. Adult female.** Body elongate, somewhat broadened toward posterior end, about 1.4–6.0 mm long, red in life. Derm membranous; mouthparts absent. Antennae, placed together on apex of head but not contiguous; usually with 9 segments, basal 2 segments much enlarged, apical segment ovoid, with a group of setae at apex, intermediate segments broader at apex than at base. Legs of moderate size relative to body; trochanter with 4–6 campaniform sensilla on each face; tibia with a tuft of clubbed setae at apex; tarsus 1 segmented and curved; claw with one denticle and 2 acute digitules. Thoracic spiracles without disc pores in atrium; abdominal spiracles smaller, with 4–6 pairs present (0 in *K. oligostigma*), each usually with a single disc pore within atrium. Anal lobe indistinct or absent; anal ring simple, subapical on dorsum. Multilocular disc pores distributed on both surfaces, of one or 2 types, each pore with one central loculus and 5–10 outer loculi; discoidal pores sometimes present on abdominal venter.

**First-instar nymph.** Body elliptical, 0.2–0.3 mm long. Derm membranous; eyes present and mouthparts well developed. Antennae placed close together at apex of head, 6 segmented, nearly clavate in form; intermediate segments short and narrow; apical segment longest and somewhat swollen, with a small group of setae at apex. Legs short and stout; tibia and tarsus apparently fused; claw with a distinct denticle and 2 digitules, each knobbed at apex and exceeding tip of claw. Thoracic spiracles each accompanied by a single multilocular disc pore; abdominal spiracles uncertain, perhaps absent. Anal ring simple, subapical on dorsum. A marginal row of discoidal pores present on dorsum of head, prothorax and abdominal segments; apical setae well developed, about one-third length of body, also with a long seta on dorsal margin of prothorax, sometimes on margin of abdominal segment VII.

## Key to species of *Kuwania* based on adult females

(*K. minuta* Borchsenius is not included\*)

1. With 4 or 6 pairs of abdominal spiracles; on Fagaceae. . . . . 2
- Without abdominal spiracles; on Burseraceae . . . . . *K. oligostigma*<sup>#</sup> De Lotto
2. Abdominal venter with discoidal pores. . . . . 3
- Abdominal venter lacking discoidal pores . . . . . 4
3. With 4 pairs of abdominal spiracles; tibia with about 6 clubbed setae. . . . . *K. quercus* (Kuwana)
- With 6 pairs of abdominal spiracles; tibia with about 10–30 clubbed setae. . . . . *K. pasaniae* Borchsenius
4. Multilocular disc pores of 2 sizes; tibia with 4–9 clubbed setae on apex. . . . . 5
- Multilocular disc pores of one size; tibia with about 12 clubbed setae at apex . . . . . *K. rubra* Goux
5. Large type of multilocular disc pore each with 8–10 outer loculi, usually 10, small type of multilocular disc pore each with 5–7 outer loculi, usually 7 . . . . . *K. raygilli* Wu & Gullan, **sp. nov.**
- Large type of multilocular disc pore each with 7 or 8 outer loculi, usually 8, small type of multilocular disc pore each with 6 or 7 outer loculi, usually 6. . . . . *K. bipora* Borchsenius

\*We have not been able to examine specimens of *K. minuta*. There is no type material deposited in the Zoological Institute, St. Petersburg, Russia, and all types may be in the Zoological Institute of Armenia (I.A. Gavrilov-Zimin 2012, personal communication). Borchsenius (1955) described this species from Ukraine and Armenia on *Quercus* sp., but provided no illustrations. We doubt that it belongs to *Kuwania* because the original description of the adult female states that the multilocular pores have two or three central loculi, and these pores in other species of *Kuwania* have one central loculus.

<sup>#</sup>We have not seen specimens of *K. oligostigma*, but the adult female described and illustrated by De Lotto (1959) resembles a species of *Kuwania* except for the absence of abdominal spiracles. It is possible that these were difficult to see or may have been removed unintentionally during maceration and clearing of body contents.

## Redescription of *Kuwania quercus* (Kuwana)

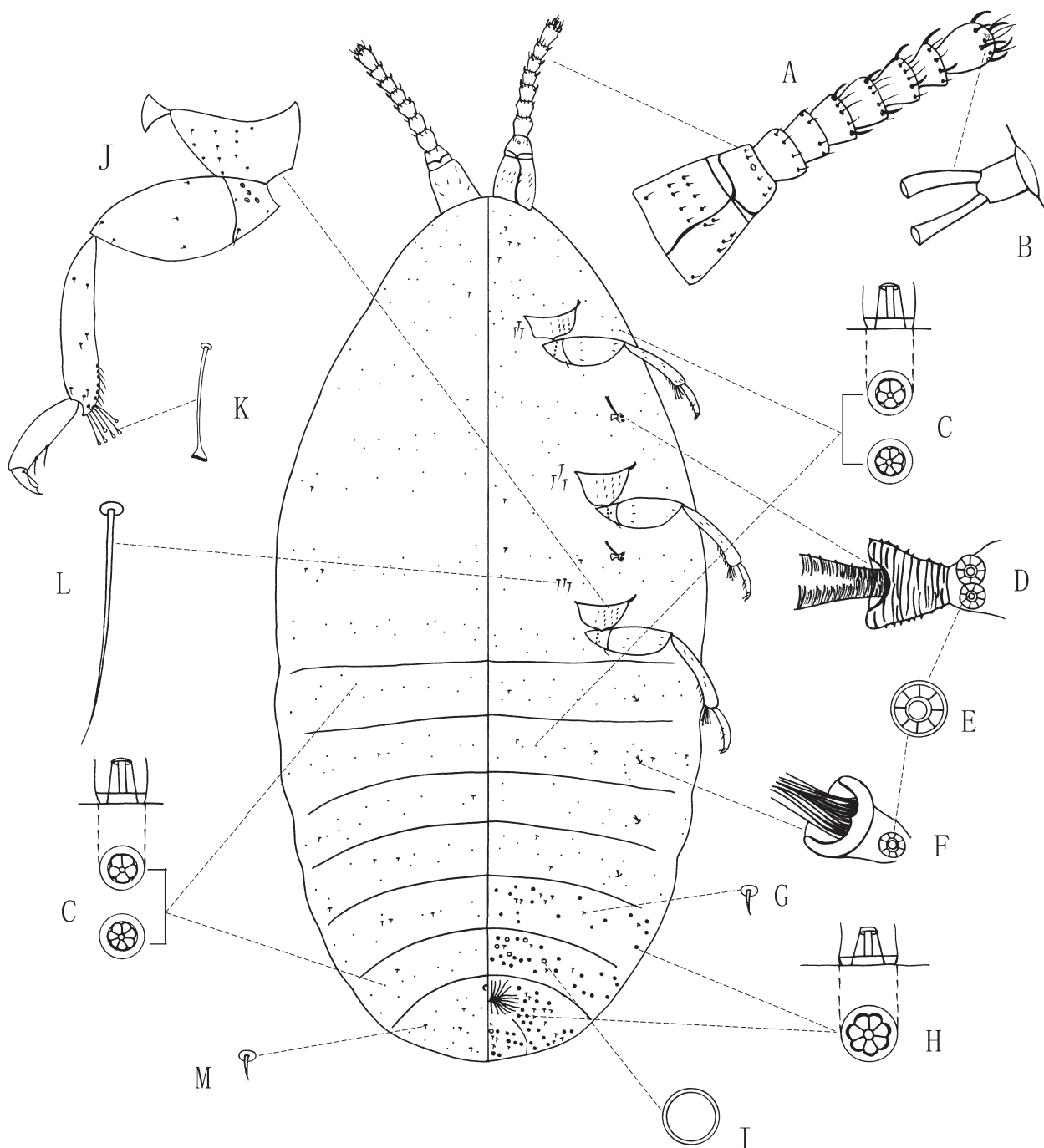
*Sasakia quercus* Kuwana, 1902: 47.

*Kuwania quercus*; Fernald, 1903: 32; Ferris, 1919: 111; Ferris, 1950: 70; Kawai, 1980: 89; Tang & Hao, 1995: 79; Ben-Dov, 2005: 56.

**MATERIAL EXAMINED.** LECTOTYPE, adult female, here designated (see notes below). Japan, date not given, Coll. Kuwana, ex *Quercus* sp., on the same slide as an adult female paralectotype, lectotype on right, paralectotype on left, slide labelled "Kuwania / quercus (Kuw) / From Oak. / Japan / Kuwana, col. / Type material / Entomological Laboratory / Stanford University" [collection data handwritten, apparently by G.F. Ferris, but name of laboratory and university is printed] (BME); PARALECTOTYPES, 12 adult females on 6 slides, 4 first-instar nymphs on 2 slides (3 on one slide, one on another slide with one adult female) and 4 intermediate-stage females on 2 slides, same data as lectotype (BME); 3 boxes with dry type material, one labelled "Kuwania quercus / (Kuwania[sic]) / TYPE / material / on Oak Quercus serrata / Kiushiu, Japan / Kuwana" [in handwriting of G.F. Ferris], another labelled "Kuwania quercus / On Quercus sp. / Tokyo, Japan Kuwana, 1900" [handwriting of G.F. Ferris] and the third box labelled "Co-type of Genus [in red ink] / Kuwania quercus. Kuwana / on Oak / Japan / Kuwana." (BME); and 5 adult females, 8 intermediate-stage females, 1 first-instar nymph and eggs (all very poor) on 2 slides labelled "CO-TYPE / 9340 / Kuwania quercus Kuw./on Quercus spp. (Katagi) = / Q. acuta / Kiushiu, Japan / Rec'd Dec. 26, 1900" (USNM).

Kuwana's original description reports that he made the collections in the year 1900 in Tokyo and Kiushiu, although he listed the hosts as *Q. myrsinaefolia* and *Q. acuta*, and did not mention *Q. serrata*. By the time that Kuwana described this Japanese species he was at Stanford University in California (as evidenced by the address on his 1902 paper) and thus Kuwana's specimens would have been available to Gordon F. Ferris, who worked on Coccoidea at Stanford University from 1917 to 1958 (Wiggins, 1958; McKenzie, 1959). The Ferris collection of Coccoidea was transferred to the BME in 1960 (Miller *et al.*, 1969), which explains how the type specimens of *S. quercus* came to be deposited there.

The International Commission on Zoological Nomenclature (1999) requires lectotype designated after 1999 to "contain an express statement of deliberate designation" (amended Article 74.7.3). We use the statement 'here designated' to satisfy this requirement. A lectotype has been designated for *Sasakia quercus* Kuwana to provide stability of nomenclature, and designation is done in a revisionary context in agreement with the amended Recommendation 74G of Article 74.7.3.



**FIGURE 1.** *Kuwania quercus* (Kuwana), adult female. A, antenna; B, a pair of coeloconic sensilla; C, small type of multilocular disc pore, showing variation in locular number; D, thoracic spiracle; E, multilocular disc pore in atrium or at opening of spiracle; F, abdominal spiracle; G, short ventral seta; H, large type of multilocular disc pore; I, discoidal pore; J, hind leg; K, knobbed seta; L, long ventral seta; M, dorsal seta.

**ADULT FEMALE** (Fig. 1) (n=13)

**Unmounted material.** According to Kuwana (1902), adult female in life reddish, antennae and legs reddish brown, usually found in crevices in bark, in a white cottony secretion.

**Mounted material.** Body elongate, somewhat broadened toward posterior apex, 1.16–1.87 (lectotype 1.62) mm long and 0.62–0.98 (lectotype 0.73) mm wide. Derm membranous with segments distinct. Eyes indistinct, mouthparts wanting. Antennae placed close to each other on apex of head but without contiguous bases, usually 9 segmented; basal segment much enlarged, cone-like, with many short fine hair-like setae and a sclerotized bar on

dorsal surface; segment II trapezoidal or cylindrical, with a whorl of short fine hair-like setae and 1 or 2 campaniform sensilla apically; segments III–VIII bowl-like, constricted at base of each segment, with a whorl of long fine hair-like setae apically, segment V–VIII each also with 2 thin-walled pegs (fleshy setae); apical segment ovoid, with 5 or 6 thick thin-walled pegs (fleshy setae), 2 or 3 long hair-like setae and one pair of coeloconic sensilla at apex; basal segment longest, nearly 1/4 of total length of antennae, segment lengths (µm): I 75–83, II 38–45, III 33–38, IV 28–33, V 30–38, VI 33–38, VII 30–35, VIII 28–35, IX 43–50. In 2 of 13 adult females, antennae 10-segmented, segments V–IX each with 2 thin-walled pegs (fleshy setae) at apex. Legs moderately developed; coxa stout, with a group (10–15) of small setae on each surface; trochanter with 4 campaniform sensilla and one short hair-like seta on each surface; femur thickest segment; tibia with a tuft of about 6 clubbed setae at ventral distal end; tarsus one-segmented, curved; claw with a denticle and 2 acute digitules, about 1/2 in length of claw. Lengths (µm): fore legs: coxa 37.5–72.5, trochanter 40.0–55.0, femur 75.0–117.5, tibia 100.0–152.5, tarsus 47.5–80.0, claw 20.0–22.5; middle legs: coxa 40.0–72.5, trochanter 37.5–55.0, femur 80.0–125.0, tibia 110.0–167.5, tarsus 55.0–87.5, claw 20.0–25.0; hind legs: coxa 45.0–75.0, trochanter 37.5–60.0, femur 87.5–125.0, tibia 110.0–165.0, tarsus 57.5–87.5, claw 20.0–25.0; ratio of length of trochanter + femur to length of tibia + tarsus of hind leg 1:1.34–1.52; ratio of length of tibia to tarsus of hind leg 1.82–1.91:1. Thoracic spiracles with sclerotized bar and spiracular atrium 12.5–19.0 µm in diameter, without disc pores within, but with one or 2 multilocular pores near opening, each pore 5.0 µm in diameter, with 7 or 8 outer loculi; abdominal spiracles in 4 pairs on margin of abdominal sterna I–IV, smaller than thoracic spiracles, each with atrium 9.5–12.5 µm in diameter, with one disc pore within atrium, this pore same size and structure as that at opening of thoracic spiracles. Anal ring circular, subapical, near anterior edge of last abdominal tergum. Vulva on ventromedial abdomen between segments VII and VIII.

*Dorsum.* Disc pores of one type only, each 3.5–4.0 µm in diameter, with a rather deeply invaginated centre and 5 or 7 outer loculi; numerous, forming transverse band on each segment, more numerous toward posterior apex. Setae tiny and slender, about 3.2–6.3 µm long, scattered over dorsal surfaces.

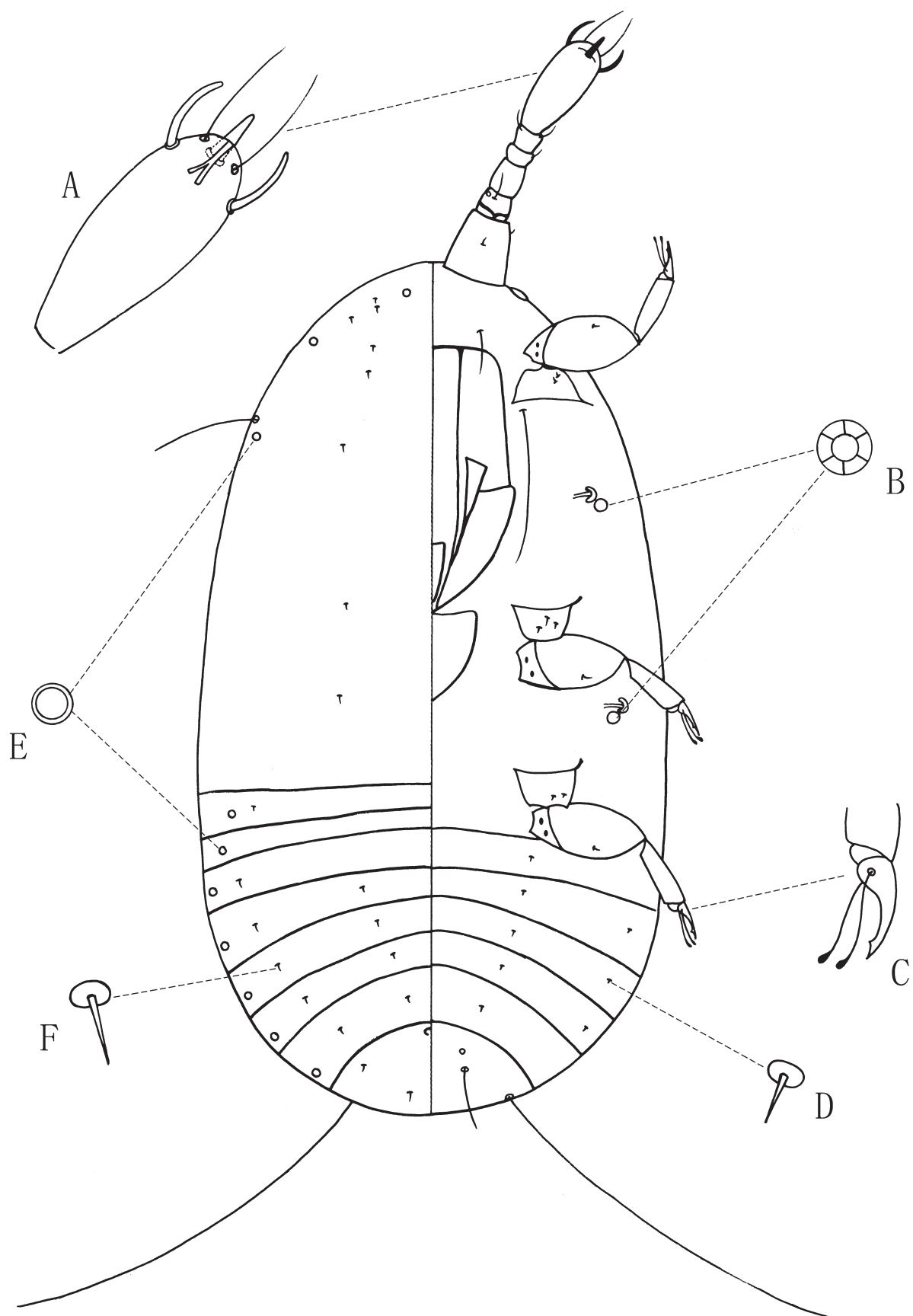
*Venter.* Disc pores of three types: (1) large multilocular disc pores, each ca. 5.0 µm in diameter, with a rather deeply invaginated centre and 7 or 8 outer loculi, mostly distributed in a large group on abdominal sterna V–VIII; (2) small multilocular disc pores, of same size and structure as those on dorsum, distributed on most of ventral surface except last abdominal segments; and (3) discoidal disc pore, each ca. 5.0 µm in diameter, about 12–18 in number, located on segments VI and VIII around vulva. Setae of 2 sizes, larger each 43.0–63.5 µm long, a few on head and near coxae; small setae similar to those on dorsum, scattered over all ventral surfaces.

#### **FIRST-INSTAR NYMPH** (Fig. 2) (n=4)

*Unmounted material.* According to Kuwana (1902), crawler in life red, with antennae and legs pale.

*Mounted material.* Body oval, 0.24–0.27 mm long and 0.13–0.15 mm wide. Eyes present, round and prominent, 3–4 µm in diameter. Mouthparts well developed, with long stylets, labium 2-segmented. Antennae close together at base, 6-segmented, 75 µm long, apex club-shaped; basal segment large and cone-like, segment III cylindrical, segment II narrow and ring-like, with 1 campaniform sensillum; segments IV and IV also narrow and ring-like; apical segment broadest and longest, elongate ovoid, with 3 thin-walled pegs (fleshy setae), 2 or 3 long hair-like setae and one pair of coeloconic sensilla at apex. Length of antennal segments (µm): I 19, II 7, III 9, IV 5, V 6, VI 29. Legs developed, thick and short; trochanter with 2 campaniform sensilla on each surface; femur enlarged; tibia and tarsus fused; claw thick and curved, with a subapical denticle; a pair of knobbed claw digitules longer than claw. Lengths (µm): fore legs: coxa 11, trochanter+ femur 25–30, tibia + tarsus 19–22, claw 9–10; middle legs: coxa 10–12, trochanter+ femur 25–31, tibia + tarsus 19–21, claw 9–10; hind legs: coxa 10, trochanter+ femur 27–31, tibia + tarsus 20–22, claw 10–11; length of trochanter + femur nearly equal to that of tibia + tarsus. Thoracic spiracle without sclerotized bar and with no disc pores within atrium; abdominal spiracles apparently absent. Anus circular, subapical, located dorsally on anterior part of last abdominal segment. Posterior end of body with a pair of long hair-like apical setae, each 98–112 µm long, about half length of body.

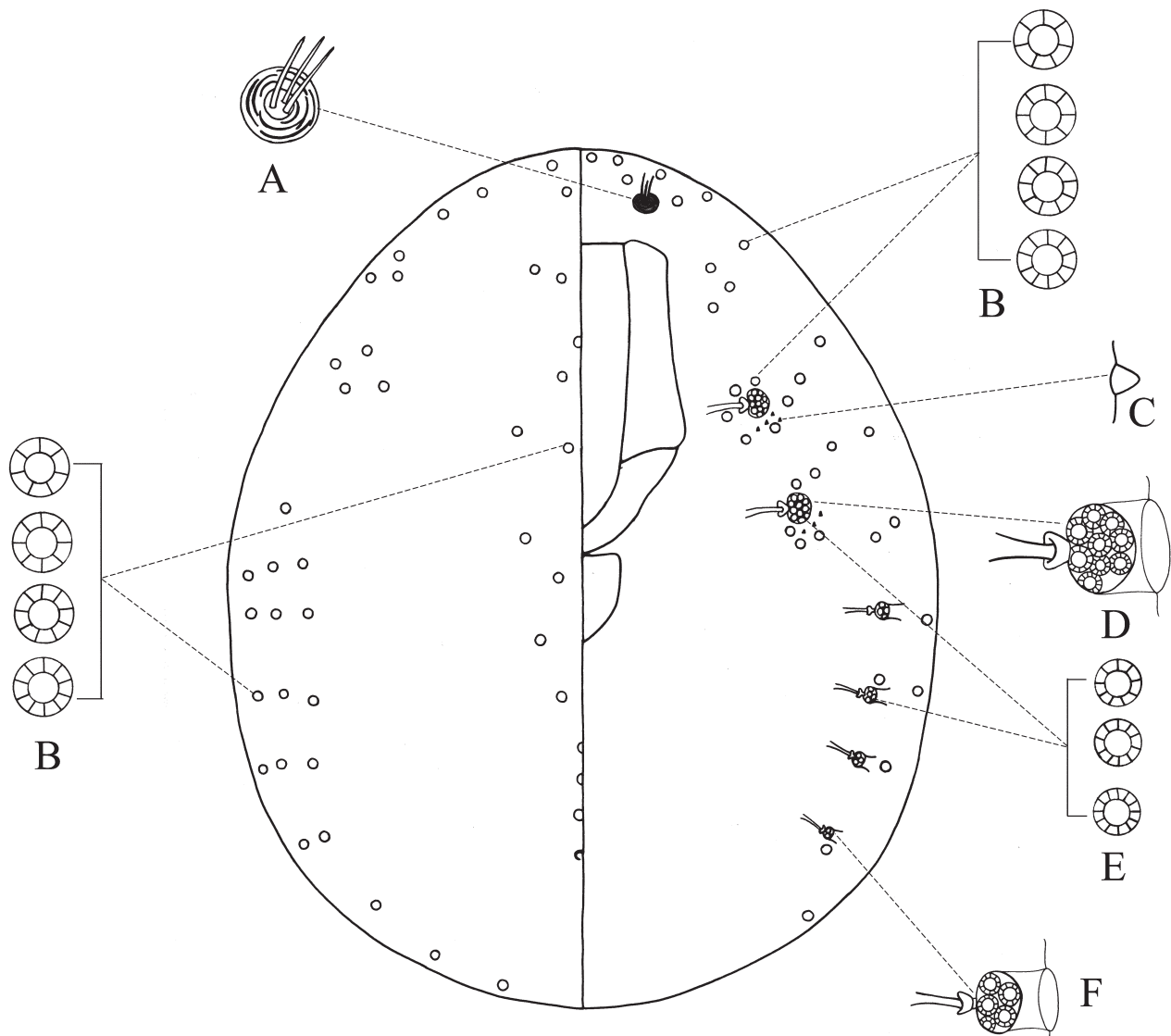
*Dorsum.* Prothorax with one pair of long hair-like setae on margin, each 34–41 µm long. Short setae, each 4.0–5.0 µm long, forming submedian longitudinal rows on dorsal surface and submarginal longitudinal rows only on abdomen. Disc pores of one type: discoidal pores each about 5 µm in diameter, 20 in number, on margin of dorsum, distributed as follows: head with one pair near base of antennae; prothorax with 2 pairs, abdominal segments I–VII each with one pair.



**FIGURE 2.** *Kuwania quercus* (Kuwana), first-instar nymph. A, apical segment of antenna; B, multilocular disc pore; C, claw of hind leg; D, short ventral seta; E, discoidal pore; F, short dorsal seta.

*Venter*. With 3 pairs of long hair-like setae: one pair in front of mouthparts, each 10–14  $\mu\text{m}$  long; one pair submedially on venter behind coxa of fore leg, each 46  $\mu\text{m}$  long, and a pair of short hair-like setae, 14–20  $\mu\text{m}$  long on submedian ventral surface of last segment. Short setae, each 4.0–6.0  $\mu\text{m}$  long, forming submarginal and submedian longitudinal rows on abdomen. Disc pores of 2 types: (1) multilocular disc pores, each with 6 outer loculi and 3.5–4.0  $\mu\text{m}$  in diameter, 4 in number, with one near each thoracic spiracle; (2) discoidal pores, each about 1.5  $\mu\text{m}$  in diameter, only 2 in number, present in front of long hair-like setae on submedian ventral surface of last segment [This kind of simple disc pore was illustrated as a hair-like seta in figure 18A of Ferris (1919) and we also initially considered it to be a broken seta (based on only one individual), but we changed our opinion after examining and finding no setae in this position on 11 individuals of *Kuwania raygilli* **sp. nov.**].

**INTERMEDIATE-STAGE FEMALE, PROBABLY THIRD-INSTAR** (Fig. 3) (n=4)



**FIGURE 3.** *Kuwania quercus* (Kuwana), intermediate-stage female. A, antenna; B, multilocular disc pores; C, conical seta with round tip; D, thoracic spiracle; E, multilocular disc pores in atrium of spiracle; F, abdominal spiracle.

**Unmounted material.** According to Kuwana (1902), body in life red.

**Mounted material.** Body broadly oval, almost circular, ca. 0.39–0.86 mm long, 0.30–0.75 mm wide, derm more or less sclerotized, with indistinct segmentation. Eyes absent. Mouthparts developed, labium 2-segmented. Antennae reduced to small plate-like structures, each with 3 short thin-walled pegs (fleshy setae). Legs wholly absent. Thoracic spiracles each with atrium 6.3–7.9  $\mu\text{m}$  across and each with 9–12 multilocular pores in atrium; each atrial pore 3.5–4.0  $\mu\text{m}$  in diameter and with 9, 10 or 12 (mainly 10) outer loculi. Abdominal spiracles in 4 pairs, one on each of anterior 4 abdominal segments, smaller than thoracic spiracles, each with atrium 4.0–5.6  $\mu\text{m}$

across and with 5–7 multilocular disc pores (like those of thoracic spiracles) in atrium. Anal ring circular, located subapically on dorsum.

**Dorsum.** Setae absent. Multilocular disc pores of one type, 5.0–6.0  $\mu\text{m}$  in diameter, with 7 to 10 (mainly 8 or 9) outer loculi, numerous, mainly forming a broad longitudinal band along margin, and a longitudinal band medially on dorsum, each of these 3 bands narrower and with fewer pores towards posterior end of body, but number and distribution of this type of disc pore quite variable, even different between 2 halves of one specimen.

**Venter.** Derm with almost no setae, only a small group (3–5) of small conical setae with round tip, located just behind opening of each thoracic spiracle. Multilocular disc pores similar to those as on dorsum, forming a longitudinal marginal band, the number also quite variable, even different between 2 halves of one specimen.

**Remarks.** *K. quercus* has been found only in China and Japan, and it appears to be an east-Asian species. There are two other *Kuwania* species recorded from Asia, i.e., *K. bipora* and *K. pasaniae*. *K. quercus* differs from *K. bipora* by the adult female having 4 pairs of abdominal spiracles and discoidal pores on the abdominal venter, and by the first-instar nymph having a discoidal pore on margin of abdominal segments II–IV and a pair of long hair-like setae behind the coxa of each fore leg, and by the intermediate-stage female lacking trilocular pores near the opening of the abdominal spiracles. *K. quercus* differs from *K. pasaniae* by adult female having 4 pairs of abdominal spiracles and each tibia having about 6 clubbed setae.

### ***Kuwania raygilli* Wu & Gullan, sp. nov.**

*Kuwania quercus* (Kuwana); Gill, 1986: 228; 1990: 62; 1991: 12; 1993: 38–39. [misidentification]

**Material examined.** HOLOTYPE: adult female, USA: California, Yolo County, Davis, University of California campus, near Academic Surge Building on the bark of *Quercus lobata*, 2 May 2012, Coll. R.J. Gill (BME). PARATYPES: 5 adult females (on 2 slides) and 9 intermediate-stage females (on 3 slides), same data as holotype (2 adult females on 1 slide and 5 intermediate-stage females on 2 slides in BFUC, 3 adult females on slide with holotype and 4 intermediate-stage females in BME); 1 adult female (DNA voucher Kuw1 of L.G. Cook), USA: California, roadside near Lake Berryessa, under bark of *Quercus* sp., 6 November 1998, P.J. Gullan (ANIC); 1 adult female (DNA voucher LGC00351), USA: California, Yolo County, Davis, UCD campus, W of Academic Surge Building, ex trunk of *Quercus lobata*, June 2005, T. Kondo (ANIC); 7 adult females (on 3 slides), 3 intermediate-stage females (on 2 slides) and many first-instar nymphs (on 4 slides), USA: California, Yolo County, Davis, UCD campus, W of Academic Surge Building, on the bark of *Quercus lobata*, 9 July 2001, Coll. P.J. Gullan (3 in ANIC, 1 in BJUC, 4 in BME and 1 in USNM).

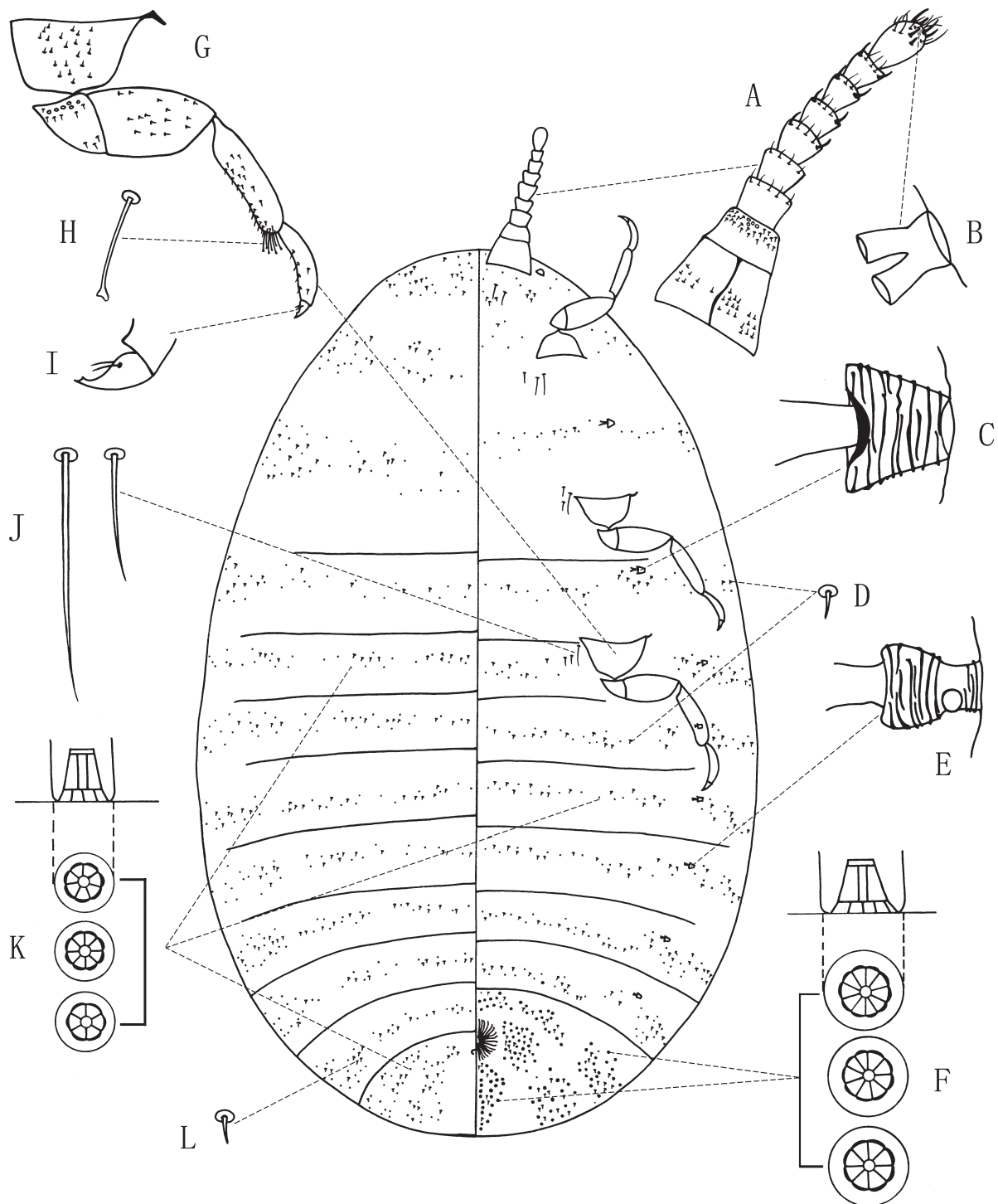
**Etymology.** This new species is named in honour of Raymond J. Gill, who first described and illustrated this species and provided specimens for our project.

#### **ADULT FEMALE** (Fig. 4) (n=9)

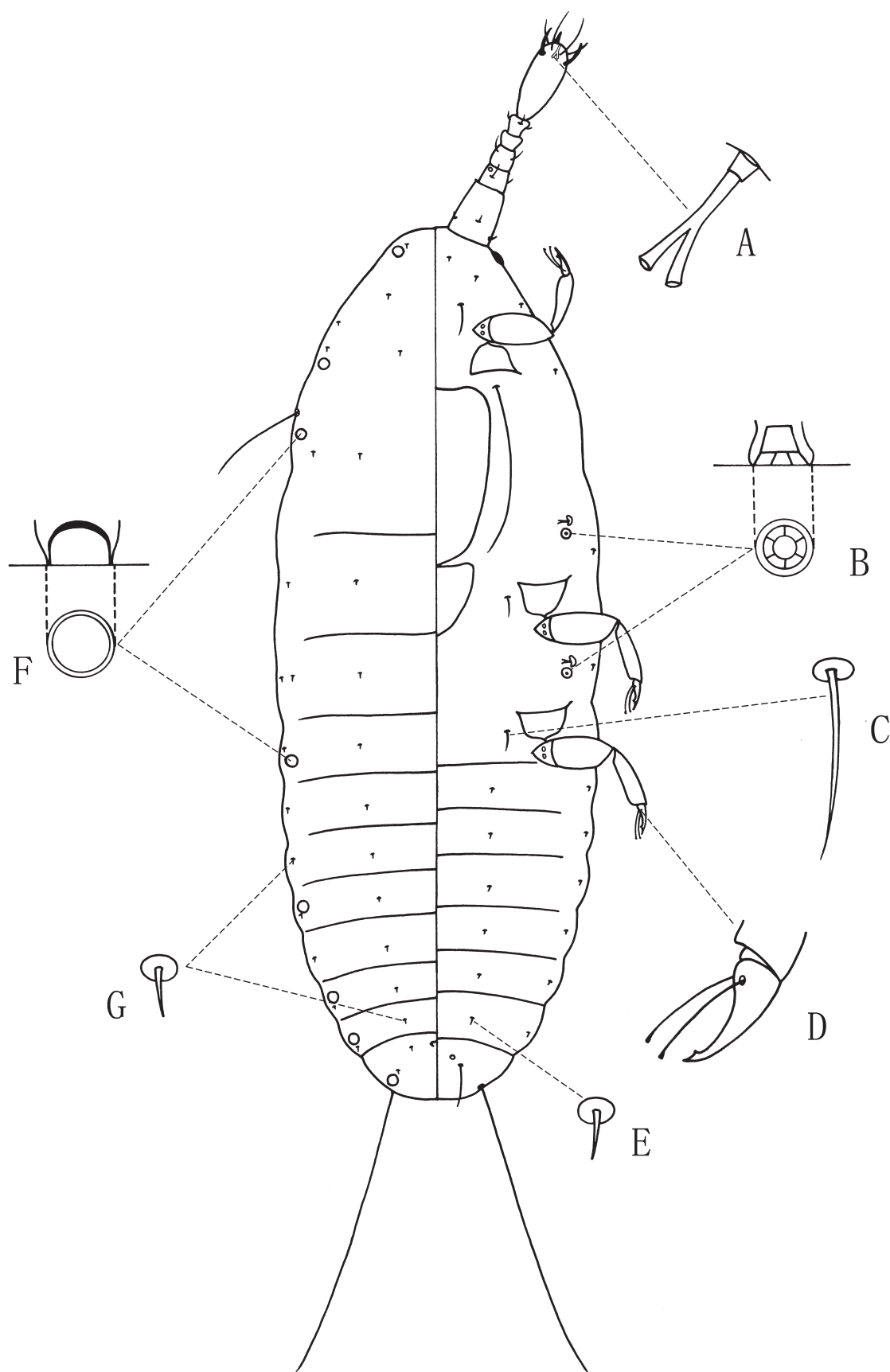
**Unmounted material.** Bright red, with a dorsal mass of white waxen threads (Gill, 1993).

**Mounted material.** Body elongate-oval, somewhat broadened toward posterior apex, 1.58–2.78 (holotype 2.78) mm long and 1.05–1.78 (holotype 1.78) mm wide. Derm membranous with segments distinct. Eyes present, mouthparts wanting. Antennae placed close together on apex of head but without contiguous bases, 9 segmented; basal segment much enlarged, cone-like, with many short fine hair-like setae in middle part and a sclerotized bar on dorsal surface; segment II trapezoidal or cylindrical, with a 3- or 4-seta wide band of short fine hair-like setae and 3 or 4 campaniform sensilla apically; segments III–VIII bowl-like, constricted at base of each segment, with a whorl of long fine hair-like setae apically, segment V–VIII also each with 2 thin-walled pegs (fleshy setae); apical segment ovoid, with 5 or 6 thin-walled pegs (fleshy setae), about 6 long hair-like setae and one pair of coeloconic sensilla at apex; basal segment longest, nearly 1/4 total length of antennae; segment lengths ( $\mu\text{m}$ ): I 95–133, II 38–65, III 28–45, IV 28–43, V 30–45, VI 30–40, VII 30–40, VIII 28–40, IX 50–75. Legs moderately developed; coxa stout, with a group of 20–25 small setae on each surface; trochanter with 4–8 campaniform sensilla, arranged in group or in line (of 100 trochanters examined, 62 with sensilla in group and 38 in a line) and 7–10 short hair-like seta on each surface; femur thickest segment; tibia with a tuft of about 7–11 clubbed setae at ventral distal end; tarsus one-segmented, curved; claw with a denticle and 2 acute digitules, each about 1/2 length of claw. Lengths ( $\mu\text{m}$ ): fore legs: coxa 58–90, trochanter 40–73, femur 98–148, tibia 115–168, tarsus 70–103, claw 25–35; middle legs: coxa 63–100, trochanter 50–73, femur 100–153, tibia 128–195, tarsus 75–115, claw 28–38; hind legs: coxa

68–105, trochanter 45–70, femur 108–155, tibia 138–218, tarsus 80–120, claw 28–38; ratio of length of trochanter + femur to length of tibia + tarsus of hind leg 1:1.43–1.55; ratio of length of tibia to tarsus of hind leg 1.71–1.89:1. Thoracic spiracles with sclerotized bar and with spiracular atrium 13–18  $\mu\text{m}$  in diameter; atrium without disc pores; abdominal spiracles in 6 pairs on margin of abdominal sterna I–VI, smaller than thoracic spiracles, each with atrium 9–10  $\mu\text{m}$  in diameter, with or without disc pore within atrium (of 104 abdominal spiracles examined, 61 without disc pore, 42 with one disc pore and only one with two disc pores). Anal ring circular, subapical, near anterior edge of last abdominal tergum. Vulva on ventromedial abdomen between segments VII and VIII.



**FIGURE 4.** *Kuwania raygilli* Wu & Gullan **sp. nov.**, adult female. A, antenna; B, a pair of coeloconic sensilla; C, thoracic spiracle; D, short ventral seta; E, abdominal spiracle; F, large type of multilocular disc pore, showing variation in locular number; G, hind leg; H, knobbed seta; I, claw; J, long ventral seta; K, small type of multilocular disc pore, showing variation in locular number; L, dorsal seta.



**FIGURE 5.** *Kuwania raygilli* Wu & Gullan **sp. nov.**, first-instar nymph. A, a pair of coeloconic sensilla; B, multilocular disc pore; C, long ventral seta; D, claw of hind leg; E, short ventral seta; F, discoidal pore; G, dorsal seta.

**Dorsum.** Disc pores of one type only, each about 5 µm in diameter, with a rather deeply invaginated centre and 5–8 outer loculi (usually 7 or 8); numerous, forming a transverse row or band on each segment; more numerous toward margin and posterior apex. Setae tiny and slender, each about 5–9 µm long, mainly present in multilocular pore bands.

**Venter.** Disc pores of two types: (1) large multilocular disc pores, each 6–7 µm in diameter, with a rather deeply invaginated centre and 8–10 outer loculi (usually 10), mostly distributed in a large group on abdominal sterna VII and VIII; (2) small multilocular disc pores, of similar size and structure as on dorsum, distributed over most of ventral surface except last abdominal segments. Setae of 2 sizes, larger each 22–53 µm long, a few near coxae; smaller setae same as on dorsum, scattered on all ventral surfaces.

**Remarks.** The adult female of this new species is very similar to that of *K. bipora*, but can be distinguished by having each large type of multilocular disc pore with 8–10 outer loculi (usually 10) and each small type of multilocular disc pore with 5–7 outer loculi (usually 7), whereas in *K. bipora*, each large type of multilocular disc pore has 7 or 8 outer loculi (usually 8) and each small type of multilocular disc pore has 6 or 7 outer loculi (usually 6). Despite these apparently small morphological differences, *K. raygilli* and *K. bipora* differ genetically from each other by as much as either differs from *K. quercus* (Table 2). Furthermore, *K. bipora* is known only from China and *K. raygilli* only from California.

**FIRST-INSTAR NYMPH** (Fig. 5) (n=11)

**Unmounted material.** None available.

**Mounted material.** Body oval, 0.32–0.39 mm long and 0.12–0.15 mm wide. Eyes present, round and prominent, each 8–10 µm in diameter. Mouthparts well developed, with long stylets, labium 2 segmented. Antennae close together at base, 6 segmented, 93–103 µm long, apex club-shaped; basal segment large and cone-like, segment III cylindrical, segment II narrow ring-like with 1 campaniform sensillum; segments IV and IV also narrow ring-like; apical segment broadest and longest, elongate ovoid, with 3 thin-walled pegs (fleshy setae), 4 long hair-like setae and one pair of coeloconic sensilla at apex. Length of antennal segments (µm): I 20–23, II 8–10, III 10–14, IV 7–9, V 9–10, VI 34–38. Legs developed, thick and short; trochanter with 2 campaniform sensilla on each surface; femur enlarged; tibia and tarsus fused; claw thick and curved, with a subapical denticle; with a pair of knobbed claw digitules, longer than claw. Lengths (µm): fore legs: coxa 12–14, trochanter+ femur 33–37, tibia + tarsus 25–29, claw 10–11; middle legs: coxa 12–14, trochanter+ femur 33–36, tibia + tarsus 25–28, claw 11–12; hind legs: coxa 14–15, trochanter+ femur 34–38, tibia + tarsus 30–31, claw 11–12; length of trochanter + femur is 1.2 times that of tibia + tarsus. Thoracic spiracle without sclerotized bar and with no disc pores within atrium; abdominal spiracles apparently absent. Anus circular, subapical, located dorsally on anterior part of last abdominal segment. Posterior end of body with a pair of long hair-like apical setae, each 98–125 µm long, about one-third length of body.

**Dorsum.** Prothorax with one pair of long hair-like setae on margin, each 42–48 µm long. Short setae, each 4.0–5.0 µm long, forming marginal and submedian longitudinal rows on dorsal surface. Disc pores of one type: discoidal pore each about 5 µm in diameter, 14–16 in number, on margin of dorsum, distributed as follows: head with one pair near base of antennae; prothorax with 1 or 2 pairs; abdominal segments I, IV, VI, VII and VIII each with one pair; occasionally pore missing on one side of abdominal segment IV.

**Venter.** With 5 pairs of long hair-like setae: 3 pairs on submedian area of thorax, lateral to each leg coxa and each 10–12 µm long; one pair submedially on venter behind coxa of fore leg, each 48–56 µm long, and one pair on submedian ventral surface of last abdominal segment, each 14–18 µm long. Short setae, each 5.0–6.0 µm long, forming marginal and abdominal submedian longitudinal rows. Disc pores of 2 types: (1) multilocular disc pores each 4.0–5.0 µm in diameter with 6 loculi, 4 in number, with one near each thoracic spiracle; (2) discoidal pores, each about 1.5 µm in diameter, only 2 in number, present in front of long hair-like setae on submedian ventral surface of last segment.

**Remarks.** The first-instar nymph of this new species is very similar to that of *K. bipora*, but can be distinguished by having one pair of discoidal pores on the margin of abdominal segment IV and one pair of long hair-like setae submedially on the ventral prothorax (none on *K. bipora*).

**INTERMEDIATE-STAGE FEMALE** (Fig. 6) (n=9)

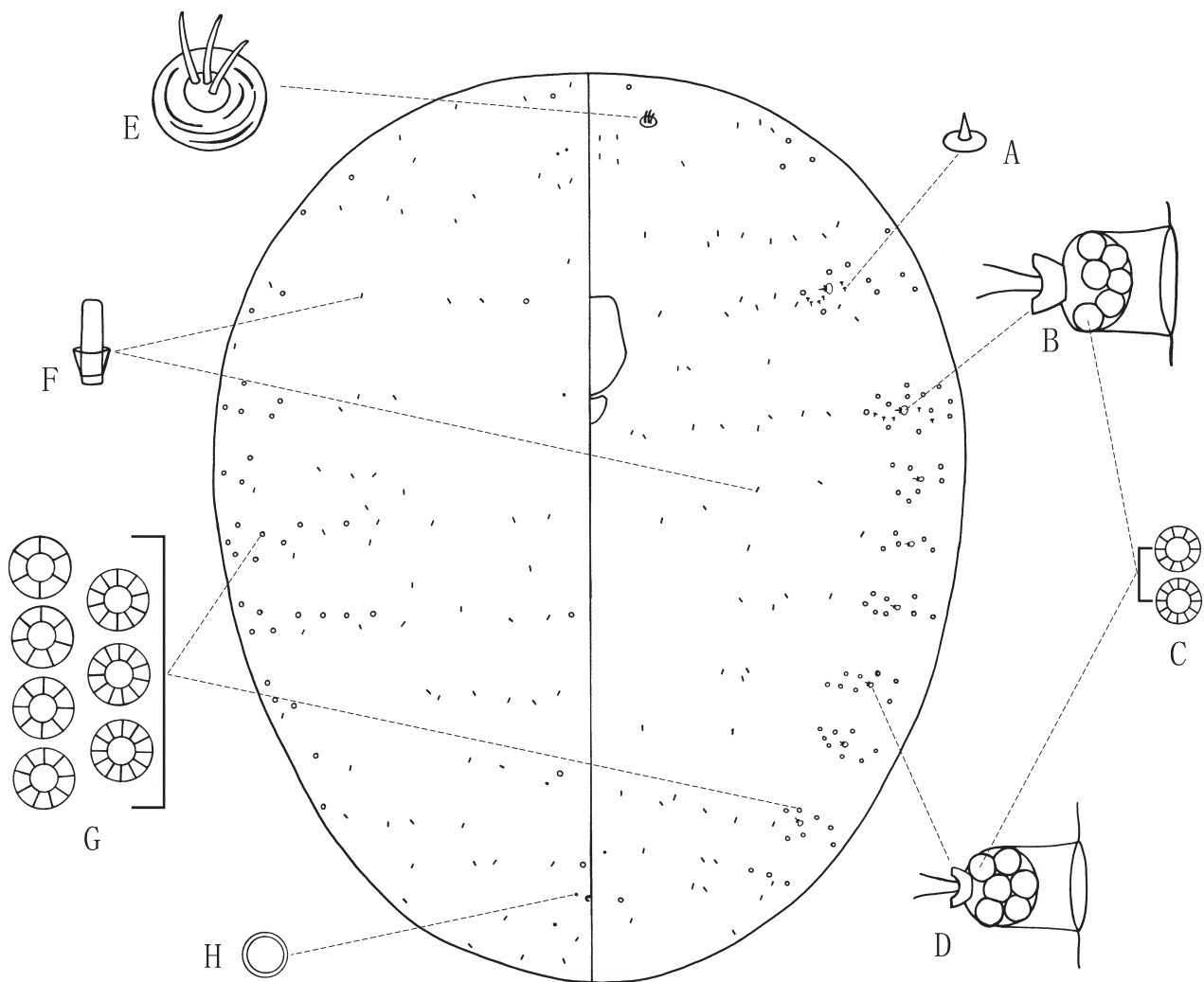
**Unmounted material.** Bright red, covered with a hard greyish or white waxen cell that blends well with colour of surrounding bark; usually hidden under rough, loosened bark (Gill, 1993).

**Mounted material.** Body on slide broadly oval, almost circular, 1.25–1.88 mm long, 1.05–1.55 mm wide; derm more or less sclerotised, with indistinct segmentation. Eyes absent. Mouthparts reduced, labium 2-segmented. Antennae reduced to small plate-like structures, each with 2 or 3 short thin-walled pegs (fleshy setae). Legs wholly absent. Thoracic spiracles, each with atrium 13–19  $\mu\text{m}$  across; atrium with 4–7 multilocular pores, each pore 5–6  $\mu\text{m}$  in diameter with 10 or 12 outer loculi. Abdominal spiracles in 6 pairs, one on each of anterior 6 abdominal segments, smaller than thoracic spiracles, each atrium 10–16  $\mu\text{m}$  across with about 4–7 multilocular disc pores (same as those of thoracic spiracles). Anal ring circular, located subapically on dorsum.

**Dorsum.** Setae absent. Disc pore of 2 types: (1) multilocular disc pores, each 6–10  $\mu\text{m}$  in diameter with 6–12 (mainly 10) outer loculi, numerous, mainly forming a broad longitudinal band along margin, each band becoming narrower and with fewer pores towards posterior end of body; also with few pores on median area of dorsum of abdomen; and (2) simple disc pores, each 4–5  $\mu\text{m}$  in diameter, scarce, only on dorsal median area of body. Microducts [Gill (1993) illustrated this gland as a discoidal pore in fig. 20A] each about 6  $\mu\text{m}$  long, 1.5  $\mu\text{m}$  wide, numerous, distributed over all dorsal surfaces

**Venter.** Derm with almost no setae, restricted to a small group of 5–7 small conical setae behind opening of each thoracic spiracle. Multilocular disc pore similar to those as on dorsum, forming a broad longitudinal band along margin. Microducts same as on dorsum, mainly present on middle area of venter.

**Remarks.** The intermediate-stage nymph is also close to that of *K. bipora*, but can be distinguished easily from the latter by the presence of numerous microducts on both surfaces of the body.



**FIGURE 6.** *Kuwania raygilli* Wu & Gullan **sp. nov.**, intermediate-stage female. A, conical seta; B, thoracic spiracle; C, multilocular disc pores in atrium of spiracle; D, abdominal spiracle; E, antenna; F, microduct; G, multilocular disc pores.

## Discussion

From their illustrations and descriptions, it is inferred that the material used by Ferris (1950) and Kawai (1980) belongs to *K. quercus*, although there are minor differences from the type material in the details of the illustrated multilocular disc pores, perhaps resulting from different interpretations or drawing styles. In contrast, the material described by Morrison (1928) and Gill (1993) is not *K. quercus*, differing mainly from the type material of *K. quercus* in the presence of six pairs of abdominal spiracles and the absence of discoidal pores on the abdominal venter the adult female. We conclude that *K. quercus* is found only in Japan, China and perhaps Korea (although we have not examined specimens from Korea), and is an east-Asian species. Furthermore, based on our morphological and molecular study, we have concluded that the Californian specimens that were described and illustrated as *K. quercus* by Gill (1993, figure 20) is a new species, that we have named *K. raygilli* Wu & Gullan. The description and illustration by Morrison (1928, figure 19) of the adult female purported to be *K. quercus* may be either *K. pasaniae* or *K. bipora*. Morrison states that his figure was based on specimens collected in 1926 but he provided no other collection data. However, the USNM holds one slide with three specimens in poor condition (two adult females and a third that also may be an adult) labelled “*Kuwanina/ quercus* (Kuw.)/ on *Quercus* sp./ Yokohama, Japan/ S. I. Kuwana, coll./ April 22, 1926.” (D.R. Miller, personal communication). These specimens may be those used by Morrison for his drawing of the adult female, but they require remounting to be able to examine their morphology clearly. In contrast, the preadult female described and illustrated by Morrison (1928, figure 20) may be that of *K. quercus* because it has four pairs of abdominal spiracles and the other features are consistent with the intermediate-stage female of *K. quercus* (as illustrated herein, Fig. 3), whereas the preadult female of *K. bipora* has six pairs of abdominal spiracles (Wu, 2008).

In the USA, the species of *Kuwanina* identified previously as *K. quercus* was found first in California along Highway 128 in Putah Creek Canyon, Yolo County, on oak in 1965, and was later collected along Highway 181 near Napa, Napa County, also on oak (Gill, 1986, 1993). In 1986, this species was collected from San Joaquin County on commercial chestnut trees and also from San Mateo County (host not specified) (Gill, 1986, 1990). In 1990 and 1991, it was found in Solano County (host not specified) and in Sonoma County on *Quercus douglasii* (Gill, 1990, 1991). To-date in the USA, this scale insect has been found only in California, where it is called Kuwana oak scale (Gill, 1993; Ben-Dov, 2005). Colour photographs and field information are provided by Swiecki & Bernhardt (2006), who report that infestations in California tend to be highly localised in distribution. In Solano and Yolo counties, adult females and nymphs of various instars have been collected in different years in every month from March to July and in November, and immature males in at least March (based on dates of collections in the California State Collection of Arthropods, CDFA, Sacramento, and those of PJG from Yolo and Napa counties). Thus, either more than one generation occurs in the warmer months, or development varies depending on climatic conditions of the collection site and year-to-year variation in temperature. Also, it is possible that adult females are long lived in California and produce offspring over an extended period. A detailed phenological study at one locality in California is required to understand the life history there. The life cycle of *K. bipora* in China has not been studied, but collection records show that adult females have been collected in late February (Borchsenius, 1960) and October (Wu, 2008).

Our taxonomic results for *Kuwanina* are significant because documenting the morphology of the type material of the type species *K. quercus* and providing an identification key to all species of the genus allows the identity of past and future collections of *Kuwanina* to be established more accurately. We have shown that *K. quercus* does not occur in the USA since populations from California that were identified previously as this species belong to a previously undescribed species most similar to the Chinese species *K. bipora*. It is possible that the Californian populations represent an exotic species to the USA, although if this is the case, the species has not been discovered yet outside of California. Individuals of *Kuwanina* are tiny (e.g., nymphs of the dispersal or crawler stage are less than 0.3 mm long) and the intermediate female stages are immobile and concealed on the bark. These inconspicuous insects would be difficult to find, even during quarantine inspections of imported plants, and quarantine restrictions were minimal decades ago. It is known that the fungal disease, chestnut blight, which affects some oak species as well as chestnut trees, was introduced to the USA around 1900 (Vossen, 2000). Plant material infested with *Kuwanina* might similarly have been introduced accidentally to California from Asia at about the same time.

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